REPEAT-BREEDER FEMALES IN BEEF CATTLE: INFLUENCES AND CAUSES[1,2]

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ABSTRACT

Straightbred and crossbred heifers (165) and cows (241) that were nonpregnant after exposure to fertile bulls for two consecutive breeding periods of 45 to 60 d (repeat breeders) and contemporary cows (102) that had produced a calf in the previous calving season (controls) were placed with bulls and were observed for mating. Repeat-breeder and control females were slaughtered at 2 to 51 d postmating. Data were accumulated over 4 yr. From previous calving records, repeat breeders had more ($P<.05$) calving difficulty than controls. Percentages of females with anatomical aberrations or anovulation were 10.9 and 3.6, and 0 and 2.9, respectively, for repeat-breeder and control females. Repeat breeders had fewer ($P<.01$) normal embryos and higher ($P<.01$) nonrecovery rates of an embryo or oocyte than controls. Precentages of normal, degenerate, unfertilized, or nonrecoveries were 42.3, 8.9, 8.0 and 40.8, and 76.8, 9.1, 6.0 and 8.1 for repeat breeders and controls, respectively. Repeat breeders had fewer ($P<.01$) 1- to 3-mm follicles than controls (25.4 and 36.5, respectively). On d 6 postmating, controls had higher ($P<.01$) serum progesterone concentrations than repeat-breeders (2.7 vs 1.8 ng/ml, respectively). Chromosome aberrations were found in lymphocytes of 19 of 133 (14.3%) repeat-breeders. Increased anatomical aberrations of the reproductive tract, increased anovulations, lower recovery rate of an oocyte or embryo, lower progesterone concentrations at d 6, fewer 1- to 3-mm follicles and increased chromosomal abnormatilities are possible causes for lower fertility in repeat-breeder females.

(Key Words: Repeat Breeders, Embryos, Ovulation, Pregnancy, Hormones, Reproductive Organs.)

Introduction

The repeat-breeder female (i.e., nonpregnant after exposure to bulls for two breeding periods of 45 to 60 d duration or after four or more artificial inseminations) and embryonic mortality have been reviewed previously (Laing, 1952; Casida, 1961; Hanley, 1961; Bane, 1964; Jainudeen, 1965; Ayalon, 1978, 1984; Sreenan and Diskin, 1983). Lower fertility in repeat-breeder females has been attributed to fertilization failure, endocrine dysfunction, increased embryonic mortality, genetic and reproductive tract aberrations. High incidences of fertilization failure have been reported in repeat-breeder heifers (Tanabe and Almquist, 1953) and cows (Tanabe and Casida, 1949). However, results from recent investigations (Ayalon et al., 1968; Ayalon, 1978; Maurer and Chenault, 1983) indicated that early embryonic mortality rather than fertilization failure was the major cause of infertility in both repeat-breeder and control females. Hormonal asynchrony also has been implicated as a cause for embryonic mortality in cattle (Shemesh et al., 1968; Pope et al., 1976; Erb et al., 1976; Maurer and Echternkamp, 1982). Gustavsson (1969) and Refsdal (1976) reported reduced fertility in daughters of bulls carrying the 1/29 chromosomal translocation. Gustavsson (1971) found that 31% of the 26 repeat-breeder heifers examined carried th

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[3]Mention of a trade name, proprietary product or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

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1/29 translocation. Also, pathology of the reproductive tract has caused infertility; Lee (1983) reported unilateral reproductive dysfunction and repeat breeding in several dairy cows.

This investigation was undertaken to determine if previous calving difficulty, fertilization failure, embryonic mortality, hormonal dysfunction, chromosomal abnormalities and(or) differences in uterine secretions were cause(s) of repeat breeding in beef heifers and cows.

Materials and Methods

Each year, approximatley 7,000 beef females (6,803 to 7,374) at the Roman L. Hruska U.S. Meat Animal Research Center were bred by either artificial insemination (AI), approximately 2,000 females, and(or) exposure to single or multiple sires in two breeding periods of 45 to 60 d duration. The breeding periods were either May 15 to July 15 or November 1 to December 31. Calf crop losses and reproductive statistics for the Research Center herd are given in table 1. During the 4 yr, 165 heifers (nonparous) and 241 cows (parous) clinically free of diseases, aged 2 to 12 yr, of various straight and crossed breeds were nonpregnant after exposure to males or AI for two consecutive breeding periods of 45 to 60 d duration (table 1). These 406 females were classified as repeat-breeders because they were nonpregnant after two consecutive breeding seasons of 45 to 60 d. Each female had the opportunity to have two to five estrous cycles per breeding period, or at least four to ten estrous cycles, to become pregnant before being classified as a repeat breeder. Palpation for pregnancy occurred at least 60 d after the end of the breeding season. Contemporary cows (102), clinically free of diseases, aged 3 to 11 yr, of various straight and crossed breeds that had produced a calf in the previous calving season served as controls.

A calving difficulty score assigned to each cow at each parturition and growth or disposal information on each calf were recorded. The scoring system for calving difficulty was: 1 — no difficulty, calved unassisted; 2 — little difficulty, assisted with hand rope pull; 3 — little difficulty with mechanical calf puller; 4 — slight difficulty with mechanical calf puller producing no injury to cow or calf; 5 — moder-

TABLE 1. CALF CROP LOSSES TO WEANING (SPRING AND FALL CALVING SEASONS COMBINED) AND NUMBER OF REPEAT-BREEDER FEMALES

Item	1979, 1980[a]	1980, 1981	1981, 1982	1982, 1983
Number females exposed to mating or AI	7,374	7,132	6,803	7,001
Percentage loss due to:				
Not pregnant at palpation	13.5	14.1	14.1	15.7
Palpated pregnant but failed to calve	1.7	2.4	3.2	2.6
Calf loss before 72 h[b]	13.4	6.2	7.1	9.1
Calf loss after 72 h[b]	1.6	1.5	2.0	3.7
Total loss	30.2	24.2	26.4	31.1
Percentage of total loss due to not pregnant at palpation	44.7	58.3	53.4	50.5
Number repeat-breeder females[c]	72	101	115	118
Percentage repeat breeder of total females exposed	1.0	1.4	1.7	1.7
Percentage of females not pregnant at palpation classified as repeat-breeders	7.2	10.0	12.0	10.7

[a]Year females exposed, year calved.

[b]Time calves died after parturition.

[c]Repeat breeder was a female not pregnant after two consecutive breeding seasons of 45 to 60 d duration. Palpation was conducted at least 60 d after the end of the breeding season. All females had the opportunity to have two to five estrous cycles/breeding season, or at least four to ten estrous cycles, to become pregnant before being classified as a repeat breeder.

ate difficulty with mechanical calf puller producing minor injury to cow or calf; 6 – major difficulty with mechanical calf puller producing severe hiplock and taking more than 30 min for delivery; 7 – caesarean birth; 8 – abnormal presentation or posture of the calf. Percentage parturition difficulty was calculated by counting the number of calving difficulty scores of 3 or more and dividing by the number of parturitions per cow multiplied by 100. Percentage abnormal presentation posture was calculated by counting the number of calving scores of 8 divided by the number of parturitions per cow multiplied by 100. Percentage calves weaned equalled number of calves weaned divided by total calves born per cow multiplied by 100. Percentage calving efficiency equalled number of calves per cow divided by cow age minus 1 multiplied by 100. Therefore calving difficulty, weaning percentage and calving efficiency were calculated for each parous repeat-breeder (241) and control (102) female from their previous calving records.

Upon being classified as a repeat-breeder, those females with palpable anomalies of the reproductive tract were slaughtered. The remaining repeat-breeders and controls were placed with multiple sires of either the Charolais or the Simmental breed and multiple-mated 30 to 45 d after palpation for pregnancy from the previous breeding season. All females were observed for estrous behavior at 0700 to 0900 and 1600 to 1800 h daily. The repeat-breeder and control females were slaughtered on d 2 to 51 postmating (estrus = d 0) and reproductive tracts collected, placed on ice and returned to the laboratory for anatomical examination and pregnancy determination. Preimplanted and early-attached embryos were flushed from the oviduct and uterine horn ipsilateral to the corpus luteum (CL) using 30 ml of saline (.9 g NaCl/100 ml H_2O) or phosphate-buffered saline (Dulbecco and Vogt, 1954). If no oocyte or embryo was recovered, the tract was flushed a second and third time with 30 ml of medium. Initially, the contralateral horn was also flushed with medium but no oocyte or embryo was ever found and this was discontinued in the last 3 yr. After thoroughly searching the flushings for either an oocyte or embryo and finding neither, the female was designated as "non-recovery" of either an oocyte or embryo. Upon finding an oocyte or embryo, it was examined for fertilization and(or) morphological development and classified as normal developing embryo, degenerate or degenerating embryo (unequal-sized blastomeres, retarded cleavage, broken blastomeres, pyknotic and shrunken blastomeres), or unfertilized oocyte (no indication of cleavage, only one polar body, no spermatozoa in zona pellucida). Fetuses 25 d or older were dissected from the uterine horn and examined for normal development (clear amniotic fluid, fetus firm with coloration).

The recovered volume of the first uterine flushing of 172 repeat-breeder and 38 control females was recorded, centrifuged at 1,050 × g for 30 min, supernatant decanted and frozen for determination of protein, calcium, zinc, and progesterone (P_4) content. Uterine flushings were from females 25 d or less postmating. Protein content of uterine flushing was determined using the fluorescamine fluorometric assay (Udenfriend et al., 1972; Böhlen et al., 1973). Calcium and zinc content were determined directly with an atomic absorption spectrophotometer using wavelengths of 2,139 for zinc and 4,227 for calcium. The ovaries were weighed and follicles were counted and recorded according to size (1 to 3, 4 to 7, and >8 mm diameter). Corpora lutea were dissected from the ovaries and weighed.

A 25-ml blood sample was collected from a subpopulation of 82 repeat-breeder and 42 control females at either d 3, 6 or 9 postmating for P_4 determination. Most of these blood samples were collected at slaughter from females either at d 3, 6 or 9 postmating. Six repeat-breeder and five control females were randomly selected, observed continuously for estrous behavior and mated to two bulls. Blood samples were collected every 4 h for 48 h beginning at onset of estrus and thereafter every 12 h until slaughter at d 8 to 10. All blood samples were immediately refrigerated at 5 C, allowed to clot, and serum was harvested within 24 h. The serum collected on either d 3, 6 or 9 or from estrus to d 8 to 10 was analyzed by radioimmunoassay for either P_4, estradiol-17β (E_2-17β) or luteinizing hormone (LH). The P_4 content of uterine fluid was also determined in flushings from 153 repeat-breeder and 29 control females.

Serum and uterine flushings, P_4 concentrations and content were determined by the single antibody charcoal radioimmunoassay for P_4 described by Maurer and Echternkamp (1982), using a specific P_4 antibody prepared against progesterone-11α-bovine serum albumin[5]. Serum and uterine flushing P_4 were extracted from .2 ml serum or 6 ml of uterine flushings. The lower limit of sensitivity of the assay for P_4 was 10 pg/tube. The intraassay coefficient of variation (CV) was 5.4% and interassay CV was 12.1% based on serum containing 1.7 ng/ml P_4 and 12 determinations.

Estrogens were extracted with 7 ml benzene from 3 to 4 ml serum, to which 1,500 CPM of NET-517 estradiol[6] was added to adjust for recovery losses. The serum was extracted twice and each extract was dried under nitrogen in a block heater (40 C). The E_2-17β content was assayed by a radioimmunoassay procedure described by Kesler et al. (1977) using a specific antisera provided by Dr. Norman Mason[7]. Crossreactivity of the antisera to various estrogens has been reported by Kesler et al. (1977). The sensitivity of this assay for 4 ml of serum was 2 pg. Intraassay CV was 3.5% and interassay CV was 15.2% based on serum with 1.9 pg/ml E_2-17β and seven determinations.

Serum LH concentrations were determined by the double antibody radioimmunoassay for bovine LH as described by Niswender et al. (1969) and modified by Echternkamp (1978). The measurable range of the LH assay was from .2 to 175 ng LH/ml serum. Interassay CV was 8.7%. Serum LH concentrations are expressed as ng NIH-LH-B8/ml of peripheral serum.

A subpopulation of 133 repeat-breeder females was analyzed for chromosomal aberrations. These were mainly nonparous females from 1980, 1981 and 1982. Peripheral blood lymphocytes were incubated for 72 h at 38.5 C and cultures were prepared by adding .5 ml heparinized whole blood to 8 ml Ham's F-10 medium supplemented with 22.5% fetal calf serum, pokeweed mitogen (37.5 μg/ml), heparin (1.875 units/ml) and antibiotics (75 units penicillin and 75 μg streptomycin per ml). Colcemid (.25 μg/ml) was added 1 h before harvesting the lymphocytes. After centrifugation at 265 × g for 20 min, the red blood cells and lymphocytes were treated with a hypotonic .075 M KCl solution at 37 C for 20 min. After centrifugation at 265 × g for 10 min and supernatant aspirated, the lymphocytes were fixed in methanol and 80% glacial acetic acid (3:1). The fixation step was repeated twice. The metaphase spreads were prepared by dropping cold, fixed lymphocytes on a precleaned warm glass slide. The chromosomes were stained in 3.5 ml of .8% Giemsa stain[8] diluted with 80 ml of cold, .07 M phosphate buffer, pH 6.8. The metaphases were analyzed with a microscope at 650× or higher magnification.

The data were analyzed using chi-square analysis and least-squares analysis (Harvey, 1975). A 2 × 2 and 2 × 4 factorial chi-square analyses were used to analyze reproductive tract, calving difficulty and pregnancy status data. The least-squares analysis was used to analyze the parturition, ovarian, uterine flushing and hormone data. Listed in table 2 are the fixed and random effects for each data set analyzed by least-squares. Two-way interactions were included in most models. Two-way interactions not used in the model and all three-way interactions were included in the residual sums of squares.

Results and Discussion

The annual calf crop losses at the Roman L. Hruska U.S. Meat Animal Research Center are summarized in table 1, and are comparable with previously reported values (Wiltbank et al., 1961; Bellows et al., 1979). The loss resulting from being nonpregnant at palpation averaged 51.8% of the total calf crop reduction over the 4 yr. The number and percentage of females exposed to males or AI that were classified as repeat breeders are listed in table 1. Although the percentage classified as repeat breeders from the total females exposed to mating was low (1.0 to 1.7), the percentage classified as repeat breeders from the females that were not pregnant at palpation averaged 10. The percentage classified as repeat breeders in dairy females was slightly higher and ranged from 10.2 to 18 (Pelissier, 1970; Ayalon, 1984).

More parturition difficulties (P<.05) were found in repeat breeders than controls (tables 3 and 4). Cow age (P<.05) and dam breed, grouped by sire breed (P<.10), were important factors in percentage calving difficulty in addition to treatments (repeat breeders vs controls). However, no significant interactions were found. Neither cow age nor breed was

[5] Miles-Yeda Ltd., Israel.
[6] 2,4,6,7,16,17-^{3}H(N); New England Nuclear, Boston, MA.
[7] Eli Lilly Co., Indianapolis, IN.
[8] Fisher, St. Louis, MO.

TABLE 2. FIXED AND RANDOM MAIN EFFECTS INCLUDED IN ANALYSES OF VARIANCE MODELS[a,b]

Source of variation	Data sets: Parturition scores	Ovarian traits	Uterine flushings	D 3, 6, 9 progesterone	Repeated blood samples for P_4, E_2 and LH
Group[c]	X	X	X	X	X
Breed by sirebreed[c]	X	X	X	X	
Pregnancy status[c]		X	X	X	X
Side of CL[c]		X	X		
Cow age[c]	X				
Days postmating[c]		X	X		X
Parity[c]				X	
Cows within group[d]					X

[a]All possible two-factor interactions among main effects included in the model for each data set were also included in the model except in the uterine flushing P_4 data set, where the two-way interactions between breed X side of CL, breed X days postmating and side of CL X days postmating were not included because of insufficient numbers.

[b]Two-way interactions not used in the model and all three-way interactions were included in the residual sums of squares.

[c]Fixed effects.

[d]Random effect.

important in abnormal presentation, whereas treatments were different (P<.05; table 3); repeat breeders had a larger percentage of parturition scores of 4, 5, 7 and 8 than controls (table 4). Repeat breeders had fewer (P<.05; x^2 = 5.41, 2 df) unassisted parturitions and more caesarean births (P<.05; x^2 = 8.74, 2 df) with their first calving than controls (table 4). Although the percentage of calves weaned did not differ between groups (table 3), the trend

TABLE 3. PARTURITION DIFFICULTY, WEANING AND CALVING EFFICIENCY PERCENTAGE FROM PREVIOUS CALVING RECORDS ON REPEAT-BREEDER AND CONTROL FEMALES[a]

Item	Group: Control	Repeat breeder[b]	Residual standard deviation
Number of cows	102	241	
Parturition difficulty[c], %	10.0[g]	29.2[h]	32.41
Abnormal presentation or posture[d], %	.05[g]	.23[h]	.36
Calves weaned[e], %	87.4[g]	78.4[g]	31.89
Calving efficiency[f], %	80.2[i]	65.8[j]	14.96

[a]Least-squares means.

[b]Repeat-breeder females had both parous (241) and non-parous (165) females. Data are only for the parous females.

[c]Percentage parturition difficulty = number parturition scores of 3 or greater/number parturitions per cow X 100.

[d]Percentage abnormal presentation or posture = number of abnormal presentation or posture/number parturitions per cow X 100.

[e]Percentage calves weaned = (number calves weaned/total calves per cow) X 100.

[f]Percentage calving efficiency = (number calves per cow/cow age minus 1) X 100.

[g,h]Group means in the same row with different superscripts differ (P<.05).

[i,j]Group means in the same row with different superscripts differ (P<.01).

TABLE 4. NUMBER AND PERCENTAGE[a] OF PARTURITIONS BY CALVING DIFFICULTY SCORES

Group		Parturitions/ number cows	Calving difficulty scores[b]							
			1	2	3	4	5	6	7	8
Control[c]	1st	102/102	55 (53.9)	5 (4.8)	5 (4.9)	20 (19.6)	2 (2.0)	8 (7.8)	4 (3.8)	3 (2.8)
	All	392/102	325 (82.9)	10 (2.6)	6 (1.5)	24 (6.1)	2 (.5)	8 (2.0)	7 (1.8)	10 (2.6)
Repeat breeder[c]	1st	241/241	100 (41.5)	7 (2.9)	8 (3.3)	57 (23.6)	11 (4.6)	11 (4.6)	38 (16.2)	8 (3.3)
	All	639/241	432 (67.6)	13 (2.0)	10 (1.6)	78 (12.2)	17 (2.7)	14 (2.2)	43 (6.7)	32 (5.0)

[a]Values in parentheses are percentages.

[b]1 = calved unassisted; 2 = assist given by hand; 3 = assistance with mechanical calf puller, little difficulty; 4 = assistance with mechanical calf puller, slight difficulty, no injury to cow or calf; 5 = assistance with mechanical calf puller, moderate difficulty, minor injury to cow or calf; 6 = assistance with mechanical calf puller, major difficulty, severe hiplock, usually more than 30-min delivery; 7 = caesarean birth; 8 = abnormal presentation or posture.

[c]Data for parous females only. All control females were parous; the repeat breeders were both parous (241) and nonparous (165).

TABLE 5. REPRODUCTIVE TRACT OBSERVATIONS OF CONTROL AND REPEAT-BREEDER FEMALES AT SLAUGHTER[a]

Group	Number females	Number normal	Number anatomical aberration[b]	Number failing to ovulate
Control	102	99 (97.1)	0 (0.0)[c]	3 (2.9)[c]
Repeat breeder	393[e]	336 (85.5)	43 (10.9)[d]	14 (3.6)[c]

[a]Values in parentheses are percentages.

[b]Females with various anatomical defects that prevented the oocyte from reaching the uterus, i.e., uni- or bilateral hydrosalpinx, adhesions of the salpinx, ovaries and(or) uterus, or occluded salpinx.

[c,d]Percentages within the same column with different superscripts differ (P<.05).

[e]Thirteen reproductive tracts were lost or mutilated at slaughter.

favored the control group. Calving efficiency (number of calves divided by cow age minus 1 times 100) was higher (P<.01) in controls than repeat breeders. This would be expected because the repeat breeders did not produce a calf in their last year and many missed one or more calvings before being nonpregnant after two consecutive breeding seasons. The increased parturition difficulty in repeat-breeder cows may have been due to size, breed and age of female, sire of calf, size of pelvic area and sex of calf (Bellows et al., 1971; Laster et al., 1973) or to hormonal asynchrony, as has been found in Holstein heifers with dystocia (O'Brien and Stott, 1977). Other hormonal changes associated with maternal and paternal contributions during pregnancy have been described by Osinga (1978), how these factors influence subsequent infertility remains to be determined.

The percentage of repeat-breeder and control females having anatomical aberrations of the reproductive tract is listed in table 5. The repeat breeders had more (P<.01; x^2 = 12.2, 1 df) reproductive tract aberrations than controls. These aberrations included ovarian encapsulation, occluded oviducts, adhesions of the reproductive tract to itself and(or) to the body wall and combinations of the above. Within the repeat-breeder females, heifers had more (15.1%) anatomical aberrations than cows (8.3%; P<.05; x^2 = 4.47, 1 df). Similar findings have been reported previously (Tanabe and Casida, 1949; Tanabe and Almquist, 1953; Casida, 1961; Graden et al., 1968).

Repeat breeders had a lower (P<.01; x^2 = 43.01, 7 df) percentage of normal embryos and fetuses (42.3 vs 76.8%) and a larger percentage of nonrecovery of either an oocyte or embryo (40.8 vs 8.1%) than controls (table 6). No differences (P>.10) in normal, degenerate, unfertilized and nonrecovery were found between repeat-breeder heifers and cows. No differences (P<.10) in the rate of fertilization failure or embryonic mortality were found between the control and repeat-breeder groups. Linares (1981/82) found fewer normal developing embryos in repeat breeders (28%) as compared with control (74%) 7 to 9 d post-mating. Possibilities for increased nonrecovery of either an oocyte or embryo were 1) rapid oocyte transport through the reproductive tract, 2) oocyte recovery by the fimbria was faulty, 3) oocyte entrapment in the follicle at ovulation, or 4) the oocyte was destroyed. Collection of embryos at d 3 did not improve the recovery rate, so rapid transport through the reproductive tract does not appear to be the cause. Preliminary results of serial-sectioning of CL tissue from nonrecovery females indicate no entrapment of the oocyte within the CL tissue. However, it is possible that the oocyte might have degenerated before ovulation, making identification difficult. Tanabe and Casida (1949) found that 3 of 78 low-fertility females had an empty zona pellucida at d 3, indicating that possibly the oocyte degenerated before ovulation. Graden et al. (1968) reported no embryo recovery in 27.7% of the repeat breeders at d 3 and 17.3% of the females at d 2 to 5. Ayalon et al. (1968) reported no recovery rates of 8 and 19% for control and repeat-breeder cows slaughtered at d 3 to 42.

Ovarian and CL weights, follicle size and number of corpora albicantia by group and pregnancy status are presented in table 7. Ovarian weight was affected by days postmating ($P<.05$), side of CL ($P<.10$; 21.2 and 19.2 g for right and left ovary; 60% of the ovulations were on the right ovary) and breed ($P<.01$). No group differences ($P>.10$) were found. Corpus luteum weights were influenced by pregnancy status ($P<.05$; normal, 4.6; degenerate, 3.6 and nonrecovery, 3.9 g), days postmating ($P<.01$) and side of CL ($P<.05$; 4.4 and 3.8 g for right and left side). However, groups did not affect CL weight ($P>.10$). Foote et al. (1959) reported no differences in CL weights, P_4 concentration and P_4 content between repeat-breeder and first-service dairy heifers. Number of follicles 1 to 3 mm diameter was goverened by groups ($P<.01$) and breed ($P<.05$). The control females had more ($P<.01$) small follicles (1 to 3 mm) than repeat breeders (table 7). A positive correlation ($r = .39$, $P<.01$) between ovarian weight and number of 1- to 3-mm follicles was found using data on all females; whereas, a negative correlation ($r = -.23$, $P<.01$) was found between the number of 1- to 3- and >8-mm follicles. Number of 4- to 7-mm follicles was dominated by pregnancy status ($P<.05$; normal, 4.2; degenerate, 2.4 and nonrecovery, 3.7), days postmating ($P<.01$) and breed ($P<.10$), but not by groups ($P>.10$). Large follicles (>8 mm) were affected by days postmating ($P<.01$) and breed ($P<.05$). Number of corpora albicantia was affected by days postmating ($P<.01$) and breed ($P<.01$). Neither large follicles nor corpora albicantia were affected by group. The only ovarian difference found was fewer 1- to 3-mm follicles in the repeat-breeder females. This may indicate that the repeat-breeder females have a smaller population of vesicular follicles or that they do not have an endocrine status sufficient for oogenesis.

Total protein (24.0 vs 25.9 mg), zinc (2.1 vs 2.2 μg) and calcium (16.8 vs 19.7 μg) content of uterine flushings did not differ ($P>.10$) between the control and repeat-breeder females, but days postmating modulated protein, zinc and calcium content ($P<.05$). Protein content was positively correlated with zinc ($r = .33$, $P<.01$) and calcium ($r = .31$, $P<.01$) content. Groups, pregnancy status, side of CL and breed were not important factors ($P>.10$). These findings do not support those reported by Ayalon (1978, 1984), who found that normal cows had higher protein concentrations than repeat-breeder females. Ayalon (1978) reported that uterine flushings from cows with abnormal embryos tended to have higher zinc and calcium concentrations than flushings from cows with

TABLE 6. PREGNANCY STATUS IN CONTROL AND REPEAT BREEDER FEMALES AT SLAUGHTER[a,b]

Group	Pregnancy status	Days postmating at slaughter			
		2 to 8	9 to 16	>16[c]	Total
Control[d]	Normal embryo	11 (52.3)	6 (37.5)	59 (95.2)	76 (76.8)
	Degenerate embryo	4 (19.1)	2 (12.5)	3 (4.8)	9 (9.1)
	Unfertilized oocyte	2 (9.5)	4 (25.0)	0 (.0)	6 (6.0)
	No. embryo/oocyte recovered	4 (19.1)	4 (25.0)	0 (.0)	8 (8.1)
Repeat breeder[d]	Normal embryo	33 (27.3)	28 (25.0)	81 (78.7)	142 (42.3)
	Degenerate embryo	15 (12.4)	6 (5.4)	9 (8.7)	30 (8.9)
	Unfertilized oocyte	16 (13.2)	11 (9.8)	0 (.0)	27 (8.0)
	No. embryo/oocyte recovered	57 (47.1)	67 (59.8)	13 (12.6)	137 (40.8)

[a]Numbers in parentheses are percentages.

[b]Statistical significance of differences discussed in text.

[c]This group is skewed towards normal embryo group because most females were slaughtered at 30 to 51 d gestation. Those females not maintaining pregnancy recycled, which placed them in the 2- to 8- and 9- to 16-d groups. This tended to lower the percentage of the normal embryo group in the 2- to 8- and 9- to 16-d groups and elevate the percentage in >16-d group.

[d]Pregnancy data were not obtained on three control females (anovulatory) and 70 repeat breeders (13 tracts lost at slaughter plant, 14 anovulatory and 43 anatomical reproductive tract aberration).

TABLE 7. OVARIAN AND CORPUS LUTEUM WEIGHTS, FOLLICLE SIZES, NUMBER OF CORPORA ALBICANTIA BY GROUP AND PREGNANCY STATUS[a]

	Group								
	Control				Repeat-breeder				Residual standard deviation
Item	Normal	Degenerate	Non-recovery	Group total[b]	Normal	Degenerate	Non-recovery	Group total[b]	
Number females	75	15	6	96	113	50	89	252	
Ovarian wt, g	21.7	22.1	18.0	20.6[c]	19.8	19.9	19.7	19.8[c]	6.8
Corpus luteum wt, g	4.6	3.9	3.7	4.1[c]	4.7	3.4	4.2	4.1[c]	1.8
Total number follicles									
1 to 3 mm	36.8	45.2	35.4	39.1[c]	27.9	29.4	21.7	26.3[d]	16.7
4 to 7 mm	4.0	1.6	3.8	3.1[c]	4.4	3.3	3.6	3.8[c]	3.3
>8 (mm)	.9	.8	.6	.8[c]	1.2	.9	1.0	1.0[c]	.8
Total number corpora albicantia	2.8	3.6	3.3	3.2[c]	3.2	4.3	3.8	3.8[c]	3.0

[a]Least-squares means.

[b]The ovaries in some of the tracts were accidentally cut off at the slaughter plant in control and repeat-breeder females. No ovarian data were collected on repeat-breeder females in 1979 and on 38 in 1980.

[c,d]Group totals in the same row with different superscripts differ ($P<.01$).

normal embryos. Using embryo transfer techniques, Gustafsson and Larsson (1983) found that embryos transferred from control and repeat-breeder females to the uterine horn contralateral to the CL of synchronized, inseminated recipients that were virgin or repeat-breeder heifers did not differ in embryonic survival. They indicated that embryo morphology, rather than the type of donor, influenced embryo survival. Tanabe et al. (1984) transferred embryos from normal cows to normal or repeat-breeder recipients and found normal embryonic development at 60 d gestation to be 82 and 70%, respectively. Almeida et al. (1984b) transferred embryos from normal and repeat-breeder donors to normal or repeat-breeder recipients and found lower pregnancy rates in the repeat-breeder recipients. Unfortunately, the normal and repeat-breeder recipients received transferred embryos at two different times and any difference may be due to embryos as well as recipients. Almeida et al. (1984a) compared the ultrastructure of the uterus from normal and repeat-breeder cows and found no differences. Therefore, uterine environment does not seem to contribute to decreased pregnancy rates in repeat breeders.

Differences in P_4 content of uterine flushings between control (252.8 pg) and repeat-breeder females (107.7 pg) were not significant. Although mean content for controls was larger than the repeat breeders, sample variability was large.

Peripheral serum was analyzed for P_4 content on d 3, 6 and 9 postmating (table 8). Serum P_4 content on d 3 was similar (P>.10), but by d 6, P_4 was higher (P<.05) in control than repeat-breeder females. Serum P_4 concentration on d 9 was unaffected (P>.10) by groups, pregnancy status, parity, breed, or interactions. Linares et al. (1982) did not find any difference between P_4 concentration in females with normal compared with those with abnormal or degenerate embryos during the first 7 d, nor did they find differences between normal and repeat-breeder females. Wiltbank et al. (1956) reported an 11% increase in normal embryonic development at 34 d in repeat breeders treated with 50 or 200 mg P_4, but this was not statistically different from nontreated repeat breeders. Echternkamp and Maurer (1983) reported lower systemic P_4 concentrations in repeat-breeder than control females. We attempted to increase systemic P_4 by giving GnRH or HCG at estrus to enhance and(or) hasten CL formation and P_4 secretion; although these treatments did not increase pregnancy rate, P_4 concentrations increased in the repeat-breeder heifers. Maurer and Echternkamp (1982), using frequent blood sampling in normal cows and heifers, did find that females with a normal developing embryo had higher P_4 concentrations on d 3 and 6 than females with an abnormal embryo.

In the five control and six repeat-breeder females that were observed continuously for estrous behavior and from which frequent blood samples were collected, LH peak heights did not differ (P>.10) between controls (71.8 ng/ml), repeat breeders (94.3 ng/ml) or pregnancy status. The time from estrus to the LH peak did not differ (P>.10) between controls (13.2 h) and repeat breeders (21.3 h), but the mean interval for the controls was less than the repeat breeders. Although control females with normal embryos had a shorter interval between estrus and LH peak (3.3 h) than repeat-breeders

TABLE 8. PERIPHERAL BLOOD PROGESTERONE CONCENTRATION (NG/ML) ON DAY 3, 6 AND 9 POSTMATING[a]

	Group				Residual
Day[b]	Number female	Control	Number female	Repeat breeder	Residual standard deviation
3	40	.49[c]	68	.49[c]	.35
6	34	2.78[c]	52	1.91[d]	.94
9	30	3.25[c]	54	3.26[c]	2.54

[a]Least-squares means.

[b]Each day was analyzed separately because most of the samples came from different cows on different days.

[c,d]Means within rows with different superscripts differ (P<.05).

TABLE 9. PROGESTERONE (P_4), ESTRADIOL-17β (E_2-17β) AND E_2-17β:P_4 RATIO BEFORE AND AFTER THE LH PEAK[a]

	Group					
	Control (5)[b]			Repeat-breeder (6)[b]		
Hours	P_4, ng/ml	E_2-17β, pg/ml	E_2-17β:P_4 ratio	P_4, ng/ml	E_2-17β, pg/ml	E_2-17β:P_4 ratio
−12	.4	12.6	33.1	.6	8.0	20.4
−8	.5	14.7	74.3	.5	10.1	40.1
−4	.3	16.1	136.7	.3	16.7	114.7
LH peak (0)	.4	13.6	66.3	.5	11.4	24.6
4	.3	9.2	48.7	.4	7.3	19.1
8	.2	5.4	33.2	.3	2.1	7.8
12	.1	5.3	54.1	.3	5.9	20.3
Residualstandard deviation for time	.2	3.9	45.7	.2	3.9	45.7
Group mean	.3[c]	11.0[d]	63.9[e]	.4[c]	8.8[d]	35.3[e]
Residual standard deviation for group	.4	9.1	95.7	.4	9.1	95.7

[a]Least-squares means.

[b]Number of females are in parentheses.

[c]Group means for P_4 did not differ (P>.10).

[d]Group means for E_2-17β did not differ (P>.10).

[e]Group means for E_2-17β:P_4 ratio did not differ (P>.10).

with normal embryos (20.0 h), the difference was not statistically significant. Neither group nor pregnancy status influences LH secretion; however, time affected ($P<.01$) LH levels.

Levels of P_4, E_2-17β and E_2-17β:P_4 ratio were not influenced by group (table 9). Time influenced E_2-17β secretion ($P<.01$) and E_2-17β:P_4 ratio ($P<.05$). Controls tended to have lower P_4 and higher E_2-17β values compared with the repeat breeders, thereby producing a larger E_2-17β:P_4 ratio. This could be interpreted to mean that the repeat-breeders may be more asynchronous in their hormone secretion. Maurer and Echternkamp (1982) have demonstrated that E_2-17β:P_4 ratios were different between females with normal and abnormal embryonic development. Erb et al. (1976) also found in nonfertile dairy cows a delayed preovulatory increase in LH after P_4 decreased to less than .74 ng/ml, as well as subsequent asynchronies of P_4, LH, estrogen, and urinary E_2-17α before the day of estrus. Lukaszewska and Hansel (1980) reported higher estrogen levels between d 6 and 16 and higher P_4 between d 10 and 18 in pregnant than nonpregnant females.

In a subsample of 133 repeat-breeder females examined for chromosomal anomalies, 19 females (14.3%) had gross chromosomal aberrations. The anomalies were 10 females with the presumptive 1/29 translocation and nine females with sex chromosomes anomalies (two females with 60,XY or 60,XX/60,XY and presumed to be a form of a freemartin, other anomalies were XXX, 59,X0/60,XX). All 10 females with the 1/29 translocation were descendants of two bulls, a Red Poll and a Simmental. Swartz and Vogt (1982) sampled a similar repeat-breeder population and found 13 of 71 (18.3%) repeat-breeder and 0 of 71 control females with chromosome abnormalities. Gustavsson (1969) and Refsdal (1976) have demonstrated reduced fertility in daughters of bulls carrying the 1/29 chromosome translocation.

Estrous cycle lengths in repeat breeders appeared to be within the normal range (18 to 24 d). Reports in the literature were supportive of normal cycle lengths (Tanabe and Casida, 1949; Tanabe and Almquist, 1953) in repeat breeders.

This investigation indicates that repeat breeding occurs with a low incidence (1.0 to 1.7%) in cattle and results from several factors. From this study, the causes could be classified as: reproductive tract anatomical aberrations, 10.9%; anovulation, 3.6%; chromosomal abnormalities, 14.3%; nonrecovery of either an oocyte or embryo, 34.7% and endocrine dysfunction and other causes, 36.5%. Calving difficulties may result in a female becoming a repeat breeder or may be another indicator of hormonal dysfunction. Other causes and combination of causes could not be readily identified. If females with anatomical and chromosomal aberrations were excluded, ovarian dysfunction appeared to be associated with the remaining 74.8%. The endocrine dysfunction was related to the hormones produced by the follicles and(or) CL while the LH measured did not differ between the control and repeat-breeder population. The reduction in small follicles in repeat breeders also is evidence for an ovarian dysfunction, but pituitary factors cannot be eliminated. Uterine variables did not differ.

Our results support Ayalon's (1978) finding that most reproductive losses occurred on or before d 8, but disagree with Hawk et al. (1955) who reported that most embryonic losses occurred between 16 and 34 d. The classification of animals that recycle, however, can change the percentages within pregnancy status classification. Further studies on repeat breeders with normal embryonic development at >35 d are needed to determine the extent of embryonic losses.

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